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Risk Factors for Progression from Cytomegalovirus Viremia to Cytomegalovirus Disease after Allogeneic Hematopoietic Stem Cell Transplantation

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Cytomegalovirus (CMV) disease is a major cause of infectious complications in allogeneic hematopoietic stem cell transplantation (allo-HSCT). Although patients undergoing allo-HSCT receive prophylactic and preemptive treatment for CMV, a subset of patients experience clinically significant CMV disease. This study investigated the risk factors for progression from CMV viremia to CMV disease during or after preemptive therapy in patients undergoing allo-HSCT. Between January 2006 and August 2010, 43 patients received preemptive therapy for CMV viremia after allo-HSCT. These patients experienced 74 episodes of CMV viremia. Nine of the patients (21%) and 12 of the episodes (16%) progressed to CMV disease. Univariate analysis identified several risk factors for progression to CMV disease, including high initial viral load (P = .020), leukopenia (P = .012), and neutropenia (P = .033) at the time of detection of CMV viremia. On multivariate analysis, leukopenia remained an independent predictor (hazard ratio, 4.347; P = .045). The rate of failure to clear CMV viremia after 1 cycle of preemptive therapy was higher in the leukopenia group than in the non-leukopenia group (60.0% versus 16.9%; P = .002). This indicates that leukopenia initially documented with CMV viremia is related to lower viral response to preemptive therapy and is a notable risk factor for progression from CMV viremia to CMV disease.

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INTRODUCTION

Cytomegalovirus (CMV) disease is a major cause of infectious complications after allogeneic hematopoietic stem cell transplantation (allo-HSCT) [1]. Even with active CMV prophylaxis provided after allo-HSCT, a subset of patients experience CMV infection or reactivation of infection, and CMV-related mortality is not uncommon. The risk factors for CMV viremia have been examined in previous studies [2,3]. Known risk factors for CMV viremia after allo-HSCT include pretransplantation CMV serostatus of donor or recipient, development of graft-versus-host disease (GVHD), ex vivo or in vivo T cell depletion, and alternative donors [4-7]. CMV viremia is typically asymptomatic, but CMV disease may be accompanied by serious clinical signs or symptoms in affected organs. For example, little progress has been made in the treatment of CMV pneumonitis over the past 2 decades [3,8], and a study from the European Bone Marrow Transplant group reported only a 31% survival rate at 1 month after diagnosis [9].

There are few reports on the risk factors for progression from CMV viremia to CMV disease in patients receiving preemptive therapy. Increased understanding of these risk factors could help guide more effective or intensive therapy before the development of CMV disease. Accordingly, the present study investigated the risk factors for progression from CMV viremia to CMV disease after allo-HSCT.

PATIENTS AND METHODS

Patients

Between June 2006 and August 2010, 114 patients underwent allo-HSCT at Severance Hospital in Seoul, Korea. Among these patients, those who received

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preemptive therapy for CMV viremia were selected for analysis. CMV viremia was detected in 43 patients, who had a total of 74 viremic episodes, and these patients' medical records were reviewed retrospectively.

CMV Prophylaxis

Acyclovir (250 mg i.v. 3 times daily) was given to all patients as prophylaxis from the day of initiation of conditioning chemotherapy to day 14 after allo-HSCT, followed by oral dosing (400 mg twice daily) for 9 months. All patients received i.v. polyvalent immunoglobulin (0.5 g/kg every 2 weeks) for 3 months, then every 4 weeks for the subsequent 6 months.

CMV Monitoring and Preemptive Therapy

All patients were monitored by CMV-specific qualitative PCR. If qualitative PCR was positive, then quantitative PCR was performed to determine viral load. Patients were monitored every week for the first month, then every 2-4 weeks until the end of treatment with immunosuppressive agents or the resolution of GVHD. If the viral load exceeded 5000 copies/mL or qualitative PCR was positive in 2 consecutive tests, then preemptive therapy was started with ganciclovir 5 mg/kg i.v. every 12 hours for 14 days (a total of 28 doses). If viremia remained after 14 days of treatment, then an additional 7 days of treatment was added at the same dose. Intravenous immunoglobulin was administered concurrently.

Blood Cell Count Sampling

A complete blood cell count sampling with differential WBC count was performed at the detection of CMV viremia. Leukopenia (WBC $\leq$3000/mm^3), neutropenia (neutrophils $\leq$1500/mm^3), lymphopenia (lymphocytes $\leq$800/mm^3), anemia (hemoglobin $\leq$10 g/dL), and thrombocytopenia (platelets $\leq$75,000/mm^3) were assessed in accordance with the Common Terminology Criteria for Adverse Events (CTCAE 4.0).

Definitions

The clearance of CMV viremia was defined as 2 consecutive negative PCR analyses after preemptive therapy. A recurrent episode of CMV viremia after clearance of previous viremia was considered a new episode. Early CMV viremia was defined as viremia detected before day 100 after allo-HSCT. CMV disease was defined as confirmation of CMV by an appropriate method accompanied by documentation of signs and symptoms in affected organs in patients who had received preemptive therapy or had a positive CMV PCR result in the previous month [10,11]. CMV pneumonitis was defined as clinical symptoms of pneumonia together with computed tomography findings consistent with CMV even without detection of CMV in bronchoalveolar lavage fluid or a lung tissue sample. CMV gastrointestinal disease was defined as gastrointestinal symptoms plus histological evidence of CMV from a gastrointestinal biopsy specimen. CMV retinitis was diagnosed by the presence of typical lesions detected by an experienced ophthalmologist, without the need for isolation of CMV in the ocular fluid.

Statistical Analysis

Statistical calculations were performed using SPSS version 18.0 (SPSS Inc, Chicago, IL). Variables were compared between groups using the Fisher exact test. Cox regression hazard models were used for multivariate analysis. A P value $<.05$ was considered statistically significant.

RESULTS

Patient Characteristics

A total of 74 episodes of CMV viremia were analyzed. The median patient age was 34 years (range, 16-54 years). CMV serostatus of recipient and donor before transplantation was positive in all episodes. In all episodes, the underlying disease was leukemia (49 episodes with acute myelogenous leukemia, 24 episodes with acute lymphoblastic leukemia, and 1 episode with biphenotypic acute leukemia). The donor was an HLA-matched sibling in 19 episodes (25.7%) and an HLA-matched unrelated donor in 55 episodes (74.3%). Stem cell source was peripheral blood in the majority of cases (89.2%). The conditioning regimen was myeloablative in 35 episodes (47.3%). T cell–depleting agents were given in 9 cases (12.2%; alemtuzumab in 8 and antithymocyte globulin [ATG] in 1).

Incidence of CMV Viremia and Disease

The median time to detection of viremia in the 74 episodes was 81 days (range, 13-1240 days) after allo-HSCT. Thirty-four episodes (46%) were detected beyond day 100 after allo-HSCT. Eight patients experienced 2 episodes of viremia, 8 patients experienced more than 2 episodes, and 27 patients experienced a single episode. Of the 43 patients with detected CMV viremia, 9 (21%) progressed to CMV disease; and among the 74 epsiodes of CMV viremia, 12 cases of CMV disease developed (16%), including 2 cases of CMV pneumonitis, 3 cases of CMV gastroenteritis, and 7 cases (3 recurring) of CMV retinitis. CMV disease occurred at median of 130 days after allo-HSCT (range, 41-994 days). The median interval between CMV viremia and CMV disease viremia was 25 days (range, 0-123 days). In 5 of the 12 CMV disease episodes (42%), CMV viremia was detected

Table 1. Characteristics of CMV Viremia and CMV Disease after HSCT

Variable	
Patients with CMV viremia, n	43
Episodes of CMV viremia, n	74
Median time of onset of first CMV viremia, days (range)	49 (13-479)
Median time of onset of CMV viremia, days (range)	81 (13-1240)
Patients with more than 1 episode of CMV viremia, n/N	16/43
2 episodes, n	8
3-7 episodes, n	8
First episode of CMV viremia beyond day +100, n/N (%)	5/43 (12)
Episodes of CMV viremia beyond day +100, n/N (%)	34/74 (46)
Patients with CMV disease, n/N (%)	9/43 (21)
Episodes of CMV disease, n/N (%)	12/74 (16)
Median interval from viremia to disease, days (range)	25 (0-123)
Median time of first CMV disease onset from HSCT, days (range)	61 (13-199)
Median time of CMV disease onset from HSCT, days (range)	130 (41-994)
First episodes of CMV disease beyond day +100, n/N (%)	2/9 (22)
Episodes of CMV disease beyond day +100, n/N (%)	5/12 (42)
Disease type, n	
Pneumonitis	2
Gastroenteritis	3
Retinitis	7
Mortality related to CMV disease, n/N (%)	3/12 (25)
Pneumonitis	2/2
Gastroenteritis	1/3
Retinitis	0/7

beyond day 100 post-HSCT. The mortality rate related to CMV disease was 25% (3 CMV-related deaths among 12 CMV disease episodes). Two of the fatal cases were CMV pneumonitis and 1 case was CMV colitis; all 3 deaths were early CMV viremia cases. Incidence and mortality data are summarized in Table 1.

Risk Factors for CMV Disease

Univariate analysis was performed to compare CMV viremia-only cases and viremia cases followed by CMV disease. Comparisons of variables between the 2 groups were assessed with the Fisher exact test, considering the small sample size. Identified risk factors for progression to CMV disease included high viral load (P = .020), leukopenia (P = .012), neutropenia at the detection of CMV viremia (P = .033), and lymphopenia on day 100 post-HSCT (P = .027). The difference in initial viral load between the viremia-only group and the CMV disease group was statistically significant (median, 15,175 copies/mL [range, 263–1,225,000] versus 78,750 copies/mL [range, 155-485,000]; Mann-Whitney U test). When episodes were subdivided into those with an initial viral load ≤20,000 copies/mL and those with an initial viral load >20,000 copies/mL, the higher viral load group had a greater rate of CMV disease (25.6% versus 5.7%; P = .020). Seventeen episodes involved neutropenia or leukopenia at the time of detection of CMV viremia, and viremia occurred after bone marrow engraftment following HSCT in 16 of these episodes. The incidence of CMV disease was greater in patients with neutropenia or leukopenia at detection of viremia than in patients without neutropenia or leukopenia (44.4% versus 12.3%, P = .033 for neutropenia; 40.0% versus 10.2%, P = .012 for leukopenia). Patients with lymphopenia at day 100 post-HSCT had a higher incidence of progression to CMV disease compared with patients without lymphopenia at the same time point (33.3% versus 9.8%; P = .027).

In this cohort, previously known risk factors for CMV viremia, including donor type, alemtuzumab or ATG treatment, and acute GVHD, did not influence the risk for progression to CMV disease. In addition, age, sex, intensity of conditioning, stem cell source, early CMV viremia, high-dose steroid therapy for GVHD, recurrent CMV viremia, anemia, lymphopenia, monocytopenia, and thrombocytopenia were not significant risk factors for progression to CMV disease.

For multivariate analysis, the following covariates were chosen based on a P value <.10 by univariate Cox regression: high viral load (P = .048), leukopenia (P = .008), neutropenia (P = .065), and lymphopenia on day 100 post-HSCT (P = .024). Donor type, alemtuzumab or ATG treatment, and acute GVHD were also included in multivariate analyses as previously known risk factors for CMV viremia despite having P values >.10. Because of the strong correlation among leukopenia, neutropenia, and lymphopenia on day 100 post-HSCT, we evaluated the independent effects of these 3 variables after adjustment for covariates including the known risk factors for CMV viremia in 3 separate models. On multivariate analysis, only leukopenia at the time of detection of CMV viremia had a significant effect on progression to CMV disease (hazard ratio, 4.347; P = .045) (Table 2).

The cumulative incidence of CMV disease at 1 year post-HSCT was 42% in patients with leukopenia and 27% in patients without leukopenia (P = .006). The cumulative incidence of progression to CMV disease by 6 months after detection of CMV viremia was 57% in patients with leukopenia and 11% in patients without leukopenia (P = .004) (Figure 1).

CMV Clearance after Preemptive Therapy

In 55 episodes, CMV viremia was cleared after 1 course of preemptive therapy. However, viremia remained in 19 episodes and progressed to CMV disease in 11 of these episodes (58%). In 4 episodes, viremia cleared after an additional course of preemptive therapy, 1 episode demonstrated self-clearance, and the remaining 3 episodes resulted in death due to another infection before clearance of viremia. All 12 episodes of CMV disease progression occurred before the clearance of viremia. Eleven episodes of CMV disease failed to respond to a single course of preemptive therapy, and 1 episode progressed during preemptive therapy. Factors associated with poor CMV clearance included use of alemtuzumab or

Table 2. Multivariate Analysis of Risk Factors for Progression from CMV Viremia to CMV Disease

Variable	Relative Risk	95% CI	Corrected *P* Value*
WBC ≤3000/mm^3			
Alemtuzumab/ATG	0.944	0.23-3.38	2.808
Donor type	3.003	0.37-24.30	.909
Acute GVHD	1.018	0.32-3.25	1.137
High viral load (>20,000 copies/mL)	4.489	0.97-20.78	.165
Leukopenia	4.347	1.33-14.25	.045†
Neutrophils ≤1500/mm^3			
Alemtuzumab/ATG	0.805	0.16-4.17	2.388
Donor type	3.24	0.4-26.15	.813
Acute GVHD	1.781	0.49-6.38	2.307
High viral load (>20,000 copies/mL)	3.180	0.67-15.02	.198
Neutropenia	3.139	0.83-11.89	.408
Day +100 lymphocytes ≤800/mm^3			
Alemtuzumab/ATG	1.184	0.28-4.97	2.451
Donor type	3.089	0.36-26.28	1.053
Acute GVHD	1.423	0.42-4.86	2.697
High viral load (>20,000 copies/mL)	2.966	0.63-13.94	.321
Lymphopenia on day +100	3.477	0.98-12.27	.159

Leukopenia, neutropenia, and lymphopenia were analyzed individually because they were clearly associated with one another.
*Bonferroni-corrected *P* value for multiple comparisons.
†Statistically significant.

ATG (66.7% versus 20.0%; *P* = .007), high initial viral load (38% >20,000 copies/mL versus 11.4% ≤20,000 copies/mL; *P* = .008), leukopenia at detection of viremia (60.0% WBC ≤3000 versus 16.9% WBC >3000; *P* = .002), and lymphopenia on day 100 post-HSCT (50% absolute lymphocyte count [ALC] ≤800 versus 15.7% ALC >800; *P* = .009) (Table 3).

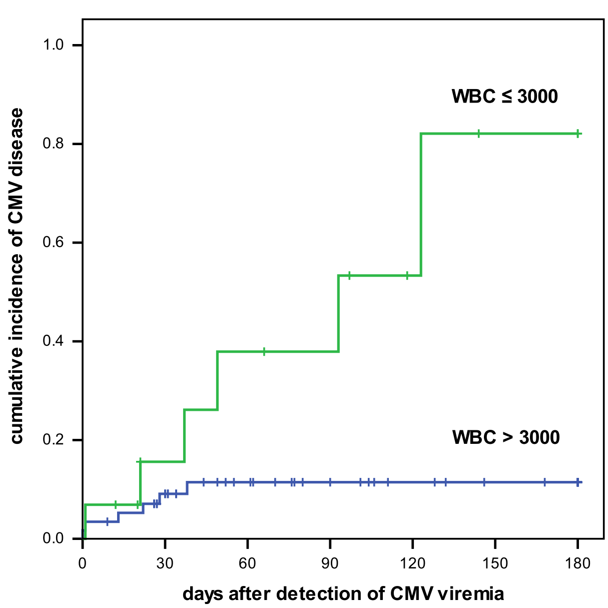

Figure 1. Kaplan-Meier estimate of the cumulative incidence of CMV disease after the detection of CMV viremia according to leukopenia. The 6-month cumulative incidence of CMV disease after the detection of CMV viremia was 57% in patients with leukopenia and 11% in patients without leukopenia (*P* = .004).

Table 3. Factors Influencing CMV Clearance after Preemptive Therapy

	Viremia Cleared (n = 55), n (%)	Viremia Remaining (n = 19), n (%)	*P* Value
CMV disease			<.001
Yes	1 (8.3)	11 (91.7)	
No	54 (87.1)	8 (12.9)	
Donor type			.127
HLA-matched sibling	17 (89.5)	2 (10.5)	
Alternative donor	38 (69.1)	17 (30.9)	
Conditioning regimen			.376
MA	28 (80.0)	7 (20.0)	
NMA	27 (71.1)	11 (28.9)	
Use of alemtuzumab/ATG			.007
Yes	3 (33.3)	6 (66.7)	
No	52 (80.0)	13 (20.0)	
Acute GVHD			.291
Present	25 (80.6)	6 (19.4)	
Absent	30 (69.8)	13 (30.2)	
High-dose steroid for GVHD			.999
Yes	13 (76.5)	4 (23.5)	
No	41 (71.9)	16 (28.1)	
Recurrent viremia			.697
Yes	26 (76.5)	8 (23.5)	
No	29 (72.5)	11 (27.5)	
Viral load			.008
≤20,000 copies/mL	31 (88.6)	4 (11.4)	
>20,000 copies/mL	24 (61.5)	15 (38.5)	
Leukopenia			.002
WBC ≤3000/mm^3	6 (40.0)	9 (60.0)	
WBC >3000/mm^3	49 (83.1)	10 (16.9)	
Neutropenia			.223
Absolute neutrophil count ≤1500/mm^3	5 (55.6)	4 (44.4)	
Absolute neutrophil count >1500/mm^3	50 (76.9)	15 (23.1)	
Lymphopenia			.062
ALC ≤800/mm^3	24 (64.9)	13 (35.1)	
ALC >800/mm^3	31 (83.8)	6 (16.2)	
Lymphopenia on day +100			.009
ALC ≤800/mm^3	9 (50.0)	9 (50.0)	
ALC >800/mm^3	43 (84.3)	8 (15.7)	
Early CMV viremia			.697
≤day +100	29 (74.3)	11 (27.5)	
>day +100	26 (74.5)	8 (23.5)	

DISCUSSION

CMV infection and CMV-related organ damage are often fatal infectious complications after allo-HSCT. Although the risk factors for CMV viremia after solid organ transplantation or HSCT have been studied extensively, little is known about the risk factors for progression to CMV disease from CMV viremia. In this retrospective study, we evaluated the risk factors for progression to CMV disease in episodes of CMV viremia occurring in patients who received preemptive therapy.

In this study, patients with initial high viral load, leukopenia or neutropenia at detection of viremia, and lymphopenia on day 100 post-HSCT had significantly higher rates of progression to CMV disease. Previous studies comparing a CMV-infected group and a noninfected group found an association between T cell immune reconstitution and CMV viremia/disease [1,4,5,12-16]. However, on univariate analysis of our data, the variables interfering with T cell immune reconstitution, including GVHD, T cell–depleting agents, alternative donors, and high-dose steroid therapy, did not have an impact on progression to CMV disease. This discrepancy might be explained by the difference between our study groups (CMV viremia versus CMV disease) and the study groups in previous reports (CMV-infected patients versus noninfected control patients).

Barron et al. [16] reported strong associations between the reconstitution of CMV-specific T cell immunity and protection against CMV viremia, clearance of viremia, and faster clearance of viremia. In our study, episodes of CMV viremia associated with lymphopenia on day 100 post-HSCT had a high rate of progression to CMV disease, and when T cell–depleting agents were involved, were associated with poor CMV clearance even after preemptive therapy. This finding suggests that the delayed reconstitution of T cell immunity is an important factor in the development of CMV disease [17], although our multivariate analysis did not demonstrate statistical significance.

Multiple studies have examined the appropriate cutoff value of CMV viral load for starting antiviral treatment [18,19]. Although some data suggest an association between higher initial viral load and higher incidence of CMV disease, there are no firm data correlating viral load with the risk for progression to CMV disease [20,21]. Our analysis divided viremia episodes into high viral load (>20,000 copies/mL) and low viral load (≤20,000 copies/mL) on the basis of a receiver operating characteristics curve analysis and examined progression to CMV disease. Similar to other reports, the group with the higher viral load had greater CMV progression.

Several previous studies have suggested an association between lymphopenia or CMV-specific T cell deficiency after HSCT and CMV reactivation [4,13,16]. Piñana et al. [15] reported that lymphopenia at day 30 and/or day 120 post-HSCT is associated with a high rate of progression to CMV disease. In our study, lymphopenia on day 100 post-HSCT was associated with CMV disease progression, and the viremia episodes with lymphopenia before day 100 had a higher incidence of progression to CMV disease (30% versus 5%; $P = .091$). However, among 74 total viremia episodes, the time of viremia detection had no statistical power, and lymphopenia at the time of viremia detection was not a significant risk factor for progression to CMV disease.

Because neutrophil and leukocyte counts are also important in hematologic malignancies and other serious infections [22], we examined the association of neutropenia and leukopenia with progression to CMV disease. Although both were found to be risk factors by univariate analysis, only leukopenia (WBC <3000 cells/mm^3) had a statistically significant effect in multivariate analysis. This finding led us to conclude that the total WBC count at the time of detection of CMV viremia is a more predictive variable than either neutropenia or lymphopenia.

Leukopenia developing after allo-HSCT has various possible causes, including the CMV viral syndrome itself. Occasionally, hematologic abnormalities such as leukopenia occur in CMV viral syndrome without the presence of atypical lymphocytes [23]. This viral syndrome may be self-limited or may progress to clinically evident organ involvement. Thus, leukopenia might not be the factor representing patient immunity, but may be the factor reflecting viral status. We confirmed that all of the 10 episodes of leukopenia recovered, within a median of 8.2 days (range, 1-24 days) after the first negative CMV PCR result after preemptive treatment.

In the present study, CMV clearance after preemptive therapy was affected by T cell–depleting agents, lymphopenia on day 100 post-HSCT, high initial viral load, and leukopenia at the time of CMV viremia detection, and more than one-half of the episodes with delayed clearance progressed to CMV disease. But although alemtuzumab/ATG therapy affected CMV clearance, it was not associated with CMV disease progression on univariate analysis, this finding might be related to the small size of our dataset.

This study has several limitations. The sample size is small, and data were collected retrospectively. Because the patients for chart review were recruited from a single transplant center, it was difficult to create a sample with a large number of patients. Further research with a larger sample size could provide more definitive evidence. In addition, there may have been patients with CMV disease who received empirical therapy without pathological confirmation. However, the relatively high statistical significance of our results suggests that underreporting of CMV disease incidence likely did not influence our findings.

In conclusion, leukopenia is a notable risk factor for progression to CMV disease from viremia, likely due to poor clearance of CMV. Thus, intensification of antiviral treatment and careful monitoring for onset of CMV disease may be warranted in patients presenting with leukopenia at detection of CMV viremia. Further prospective studies with larger patient cohorts are needed to reconfirm our findings.

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